Structure of Cow's κ-Caseino-Glycopeptide: the N-Terminal Octadecapeptide*

The primary enzymic phase of the action of rennin (EC 3.4.4.3) on casein[1] in which the protective colloïd is destroyed, has been shown to be confined to the κ-casein-fraction[2]. During this phase, rennin liberates from cow's κ-casein a glycopeptide which we called κ-caseino-glycopeptide (κ-CGP): it does not dialyze and is soluble in 12% trichloroacetic acid. The κ-CGP contains 25 to 30% carbohydrate, about 70% peptide material and about 0,4% phosphorous, with a molecular weight of 6.000–8.000[3,4]. The peptide moiety consists of 12 different amino acids and is devoid of cystine, aromatic amino acids, histidine and arginine[3,5,6,7] (Table I). The C-terminal sequences of κ-CGP as well as of κ-

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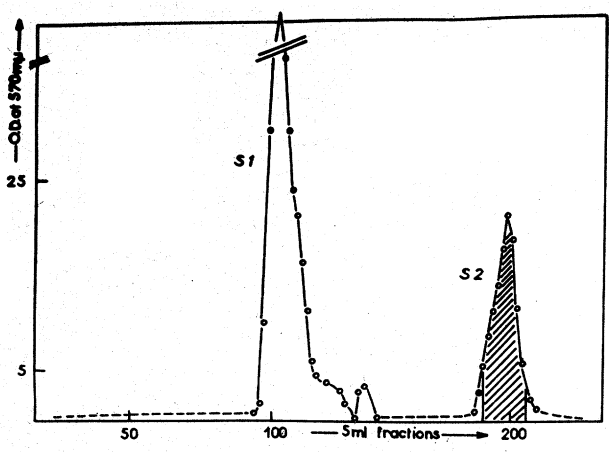
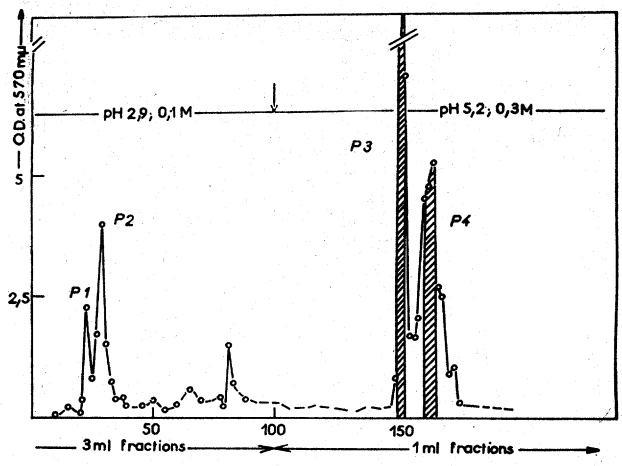
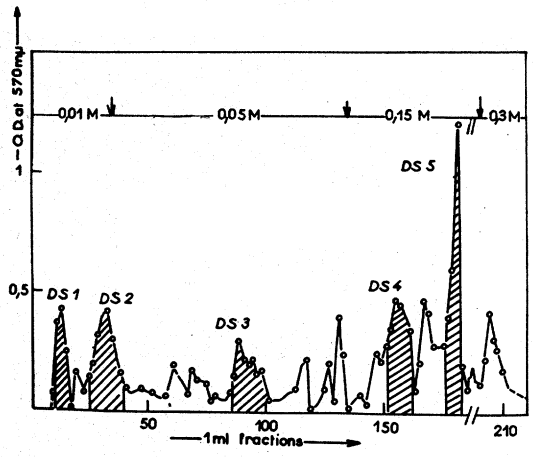


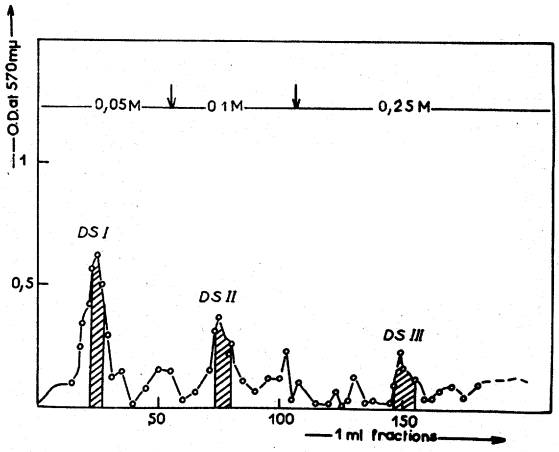
1a) Filtration on Sephadex G.25 (280 × 2.6 cm) of the chymotryptic digest of cow's κ-CGP



1b) Purification on P-cellulose (57 × 1 cm) of peak S2



1c) Chromatography on DEAE-Sephadex A-25 of the tryptic digest of peptide P3



1d) Chromatography on DEAE-Sephadex A-25 of the tryptic digest of peptide P4

Figure 1. Chromatographic separations of chymotryptic and tryptic peptides from cow's κ-CGP

casein were reported to be the same, containing valine, threonine, alanine and serine[8]; the N-terminal sequence of κ-CGP was recently reported to be H.Met-Ala-...[9]. The elucidation of the structure of the N-terminal octadecapeptide of cow's κ-CGP will be described in the present note.

140 mg of κ-CGP were submitted to a chymotryptic (EC 3.4.4.5) digestion (*p*H 7.5; 37°; 6 h; enzyme/substrate: 1/50). The enzymic hydrolysate was filtrated on a 280 × 2.6 cm column of Sephadex G-25 (fine) with water as eluent. Two peaks, S1 and S2, were characterized with ninhydrin after alkaline hydrolysis (Fig. 1a). As only peak S2 contained methionine, this latter was submitted to further purifications in view to determine the N-terminal sequence of κ-CGP.

After chromatography of peak S2 on P-cellulose (57 × 1 cm; ammonium acetate-formic or acetic acid buffers), four main fractions, P1 to P4, were obtained (Fig. 1b); only P3 and P4 contained methionine (for amino acid compositions see Table I).

P3. The N- and C-terminal sequences of P3 are respectively Met-Ala-Ileu (Edman degradation) and (Asp[NH_2], Thr, Ileu) (carboxypeptidase). This fraction was submitted to a tryptic (EC 3.4.4.4) hydrolysis (*p*H 7.5; 37°; 6 h; enzyme/substrate: 1/20) and the tryptic digest was chromatographed with ammonium bicarbonate solutions on a DEAE-Sephadex column (50 × 0.6 cm); five fractions, DS1 to DS5, were characterized (Fig. 1c).

Fraction DS1. Composition: Pro_2, Ala_1, Met_1, $Ileu_1$, Lys_2.

Fraction DS2. Composition: Pro_2, Ala_1, Met_1, $Ileu_1$, Lys_1. N-terminal sequence (Edman degradation): H.Met-Ala-Ileu. Structure: H.Met-Ala-Ileu-Pro-Pro-

[8] P. JOLLÈS, C. ALAIS and J. JOLLÈS, *Arch. Biochem. Biophys. 98* (1962) 56.

[9] A. DELFOUR, J. JOLLÈS, C. ALAIS and P. JOLLÈS, *Biochem. Biophys. Res. Comm. 19* (1965) 452.

Table I. Amino acid compositions (residues) of cow's κ-CGP and of peaks P3 and P4 (see Fig. 1b)

Amino acid	κ-CGP (this study) Hydrolysis 18 h	36 h	nearest integer	P3 nearest integer	Residual peptide after Edman degradation 1st step	2nd step	3rd step	P4 (nearest integer)
Asp	3.6	3.6	4	3	3	3	3	2
Thr	8.3	4.5	9–11	2	2	2	2	1
Ser	4.1	1.2	5–6					
Glu	9.0	9.0	9	2	2	2	2	2
Pro	6.1	6.1	6–7	3	3	3	3	2
Gly	1.0	0.9	1					
Ala	4.3	3.8	4–5	1	1			1
Val	4.1	3.9	4–5					
Met	0.8	0.0	1	1				1
Ileu	4.9	4.8	5	3	3	3	2	1
Leu	1.0	1.0	1					
Lys	2.8	2.9	3	3	+	+	+	3
Total			52–58	18				13

Table II. Chemical structure of the N-terminal octadecapeptide of cow's κ-CGP

Composition: Asp_3, Thr_2, Glu_2, Pro_3, Ala_1, Met_1, $Ileu_3$, Lys_3 (fraction P3, see Fig. 1b)

Method	Sequences
Edman	Met-Ala-Ileu
Aminopeptidase	Met-Ala
Carboxypeptidase	(Asp [NH_2], Thr, Ileu)
Tryptic units	Met-Ala-Ileu-Pro-Pro-Lys
	(Met,Ala,Ileu,Pro,Pro,Lys)-Lys
	Lys-Asp(NH_2)-(Asp*, Glu*)-Lys
	Asp -(Asp*, Glu*)-Lys
	Thr-Glu-(Pro, Ileu, Asp [NH_2], Thr, Ileu)
Structure	Met-Ala-Ileu-Pro-Pro-Lys- Lys-Asp(NH_2)-(Asp*, Glu*)-Lys-Thr-Glu-(Pro, Ileu)-(Asp [NH_2], Thr, Ileu)

* It was not determined which of these residues is aminated.

Lys. The structure of DS1 (above) was established by the same way (Table II).

Fraction DS3. Composition: Asp_2, Glu_1, Lys_2. N-terminal amino acid (Edman degradation): Lys. N-terminal sequence (amino-peptidase): Lys-Asp(NH_2). Partial structure: Lys-Asp(NH_2)-(Asp*, Glu*)-Lys.

Fraction DS4. Composition: Asp_2, Glu_1, Lys_1. The N-terminal amino acid (Edman degradation) is Asp. Partial structure: Asp-(Asp*, Glu*)-Lys.

Fraction DS5. Composition: Asp_1, Thr_2, Glu_1, Pro_1, $Ileu_2$. N-terminal sequence (Edman): Thr-Glu. Partial structure: Thr-Glu-(Pro, Ileu, Asp [NH_2], Thr, Ileu).

P4. The N- and C-terminal amino acids are respectively Met and Glu. This fraction was also submitted to a tryptic hydrolysis (*p*H 7.5; 37°; 6 h; enzyme/substrate: 1/20) and the tryptic digest was chromatographed with ammonium bicarbonate solutions on a DEAE-Sephadex column (20 × 1 cm); three main fractions, DSI to DSIII, were characterized (Fig. 1d).

Fraction DSI. Identical to fraction DS2.

Fraction DSII. Identical to fraction DS3.

Fraction DSIII. Dipeptide: Thr-Glu.

From all these results summarized in Table II, the structure of the N-terminal octadecapeptide of cow's κ-CGP was obtained (Table II). This N-terminal sequence contains all the lysine residues of the κ-CGP, its unic methionine residue, three of the four Asp residues and one half of the Pro residues; it does not contain sugars (slight basic part of κ-CGP). The carbohydrate moiety is situated in the C-terminal part of κ-CGP and this latter is contained in peak S1 (Fig. 1a) (acidic part of κ-CGP).

Finally it is noteworthy to mention that identical results were obtained with the sheep caseino-glycopeptide.

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* It was not determined which of these residues is aminated.